

# The flaws of laser Doppler in negative-pressure wound therapy research

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## Abstract

Recent studies, using modalities other than laser Doppler, have indicated that perfusion during negative-pressure wound therapy (NPWT) is reduced, contrary to world literature. The aim of the present study was to evaluate whether the measuring technique of the laser Doppler could be influenced by the compressive nature of NPWT dressings and whether this could explain the conflicting findings. A hypothesis that it may be possible for laser Doppler to record similar readings to those obtained during NPWT by merely compressing tissues manually was tested on 12 NPWT dressings, with each undergoing an alternating series of manual compressive forces and NPWT (−125 mmHg). During the periods of NPWT ( $n = 12$ ), the mean perfusion recording increased in five experiments, reduced in six, and remained unchanged in one. During the period when manual pressure was applied ( $n = 12$ ), there was a mean increase in perfusion in six experiments and a reduction in six. The type of change in perfusion (increase or decrease) was the same for both NPWT and manual pressure in 10 of the 12 experiments. In conclusion, laser Doppler can incorrectly record increased perfusion when tissues are compressed, implying that it is flawed in the field of NPWT research as tissues are always compressed to some degree by the NPWT dressing.

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Negative-pressure wound therapy (NPWT) has become an integral part of any surgeon's armamentarium when dealing with complex wounds. Numerous controversies exist regarding its mechanism of action and, as a result, confusion exists as to the correct pressure settings and type of wound interface. Recent work has even proposed that certain indications for NPWT (e.g., tissues with compromised perfusion) may actually be contraindications.<sup>1</sup>

This confusion regarding its mechanism of action may stem from the large amount of conflicting evidence regarding how NPWT affects perfusion. The common perception that "negative-pressure" wound therapy results in a pressure gradient, which causes a surge of blood flow toward the wound,<sup>2–5</sup> has recently been challenged by evidence to suggest otherwise.<sup>6–9</sup> In keeping with this evidence that NPWT increases tissue pressure, a recent study showed that both circumferential and noncircumferential NPWT dressings reduce perfusion.<sup>1</sup>

Despite the fact that NPWT increases tissue pressure and that this is incongruous with an increase in perfusion, there remains an overwhelming body of evidence that has shown an immediate increase in perfusion.<sup>2,3,5,10–22</sup> This apparent paradox has remained unexplained.

Almost all of the studies evaluating perfusion changes due to NPWT have made use of laser Doppler.<sup>10–18,20–22</sup> The laser Doppler

measurement of perfusion (perfusion units) is an arbitrary unit that is derived by multiplying the velocity and concentration of red blood cells within a tissue volume (less than 1 mm<sup>3</sup>).<sup>17,18,23,24</sup> When the light of the laser Doppler encounters a moving red blood cell, it undergoes a wavelength shift explained by the Doppler effect, while the static objects in its path do little to change its wavelength.<sup>18</sup> The wavelength changes are therefore affected primarily by the concentration and velocity of red blood cells.<sup>18</sup> It has been suggested that the velocity parameter provides a more direct measure of physiological changes than the concentration parameter.<sup>24</sup>

However, doubts have been cast on the accuracy of this modality,<sup>25</sup> particularly in the setting of NPWT.<sup>1,26</sup> A proposal was made to explain the paradoxical findings of the laser Doppler in recent work.<sup>1</sup> The proposal for the paradoxical findings was based on the continuity equation, whereby fluid in steady state flow will undergo an increase in velocity if the tube within which it is flowing undergoes a reduction in diameter. If tissues were to be compressed, as occurs during NPWT, the diameters of the vessels are reduced. This may increase the velocity of blood flowing through the narrower vessels, thereby resulting in the laser Doppler incorrectly interpreting this as an increase in perfusion. This would be analogous to compressing the end of a garden hosepipe, which results in an increased velocity of the water, despite potentially decreasing total flow.

NPWT Negative-pressure wound therapy  
VAC Vacuum-assisted closure

If this theory were true, then manual compression of tissues beneath a weight could result in partially occluded vessels (with increased blood velocities), which would be incorrectly interpreted as increased perfusion by the laser Doppler. And if one were to increase tissue pressure further, this would result in total vessel occlusion with a correct reading of reduced perfusion by the laser Doppler, as the *velocity* would also be reduced to zero for most vessels.

The increase in tissue pressure for a given weight varies depending on numerous variables, such as the consistency of the tissue and the region of the body. Therefore, according to the above theory, applying the same weight on different regions of the body would also result in different laser Doppler recordings, with some being increased and some reduced. Similarly, applying an NPWT dressing at a given suction pressure to different regions of the body would (theoretically) also result in different laser Doppler readings. These theories were tested in the current study.

## Materials and methods

The study was conducted in a room with a temperature set at 20 °C. A total of 12 vacuum-assisted closure (VAC) dressings (Kinetic Concepts, Inc., San Antonio, TX) were placed on different anatomical regions in two healthy volunteers. The slabs of foam (3.3 cm thick) were cut into squares (4 × 4 cm). In each volunteer, a VAC dressing was placed on intact skin on their dorsal forearm, anterior thigh, and on either side of the upper chest and lower back. When suction was to be applied, this was done at -125 mmHg. A laser Doppler sensor (moorVMS-LDF2, Moor Instruments, Devon, UK) was placed 1.5 cm from the edge of the foam, directly on the skin in a dedicated probe holder.

The technique of applying manual pressure to test the hypothesis was by using two weights, measuring a total of 1.75 kg. Unfortunately, there was no way of determining the exact amount of weight to apply to recreate the same tissue pressure generated by an NPWT dressing of equivalent proportions (with suction on) without invasive tissue pressure sensors. Therefore, the weight was determined in a preliminary test, whereby different weights were applied to an NPWT dressing (without suction) on a healthy volunteer's thigh.

The least amount of weight necessary to elicit a noticeable change in the laser Doppler trace on the monitor was determined to be 1.75 kg in that experiment. As the application of more weights to the dressing was difficult to balance on the dressing, this weight was used throughout the rest of the study. It has to be mentioned at this stage that the actual weight is of less significance than how the Doppler responds to a weight.

To prevent these weights sliding off the dressing, they were strapped together using tape and were placed onto a light (50 g) neoprene padded band, which could be balanced on the dressing. The total weight was, therefore, 1.8 kg. Care was taken to ensure that the neoprene band (and weights) applied pressure to the NPWT dressing only and not to the laser Doppler sensor.

The experiment was begun in all 12 studies by recording laser Doppler readings for two minutes (with the dressing in place without suction) to obtain a baseline reading. The following sequence was

then carried out on the left thigh and chest, and right forearm and back (eight sites): suction was applied (at -125 mmHg in all cases) for 2 minutes, followed by 2 minutes of no suction (to allow perfusion to revert to normal) and then 2 minutes of manual pressure with the weight (also without suction).

In order to ensure that the order of the sequence did not influence the results, an additional four experiments were undertaken on the contralateral sides of the chest and back, with the sequence reversed. In other words, after the baseline reading was taken, manual pressure was applied first (for 2 minutes), followed by 2 minutes without the weight, and then followed by 2 minutes of suction.

Because of the clarity of the results, only a qualitative data assessment was deemed necessary. The study was approved by the Human Research Ethics Committee of the University of Cape Town.

## Results

In the 12 experiments, a mean of 4,340 (standard deviation 942) perfusion measurements were recorded for each 2-minute period (baseline, NPWT, or manual pressure) tested. During the periods of NPWT at -125 mmHg ( $n = 12$ ), the mean laser Doppler perfusion recording increased in five experiments, reduced in six, and remained unchanged in one (Table 1). During the period when manual pressure (weight) was applied ( $n = 12$ ), there was a mean increase in perfusion in six experiments and a reduction in six.

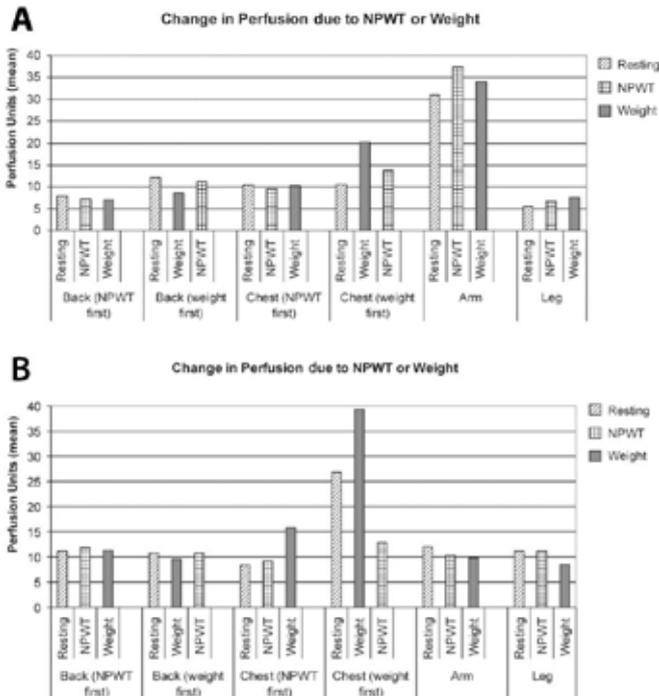
The type of change in perfusion (increase or decrease) was the same for both NPWT and manual pressure in 10 of the 12 experiments (Figure 1). In one of the other two experiments, the mean change in perfusion remained unchanged during NPWT but reduced during manual pressure application. In the other, the mean changes in perfusion were discordant during NPWT and manual pressure, with a mean increase being recorded during the application of manual pressure and a reduction during NPWT.

**Table 1.** Number of cases showing increased, decreased, or unchanged laser Doppler perfusion readings following application of NPWT or manual pressure (weight)

|                        | Laser Doppler perfusion readings |          |           |          |
|------------------------|----------------------------------|----------|-----------|----------|
|                        | Increase                         | Decrease | Unchanged | <i>n</i> |
| <b>Application of:</b> |                                  |          |           |          |
| NPWT (-125 mmHg)       | 5                                | 6        | 1         | 12       |
| Weight (1.8 kg)        | 6                                | 6        | 0         | 12       |

NPWT, negative-pressure wound therapy.

By virtue of the fact that the reversed sequences ( $n = 4$ ) had similar outcomes to the initial experiments ( $n = 8$ ), it did not appear that the sequence affected the outcome measurements. Only one of the initial experiments ( $n = 8$ ) and one of the reversed-sequence experiments ( $n = 4$ ) had changes in perfusion that were discordant in the NPWT and weight groups. Although a formal analysis to compare the two types of sequences was not done because of the small numbers, this aspect was of minimal relevance to the central hypotheses of the study.



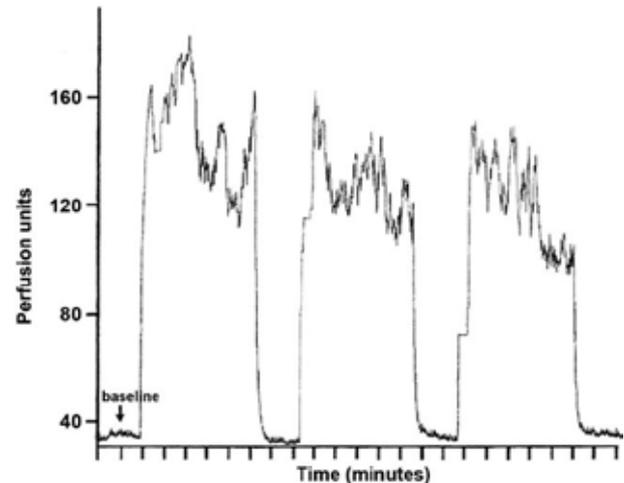
**Figure 1:** Laser Doppler perfusion changes during various sequences of NPWT at  $-125$  mmHg and manual pressure (weight 1.8 kg) in different anatomical regions in volunteers A and B. NPWT, negative-pressure wound therapy.

## Discussion

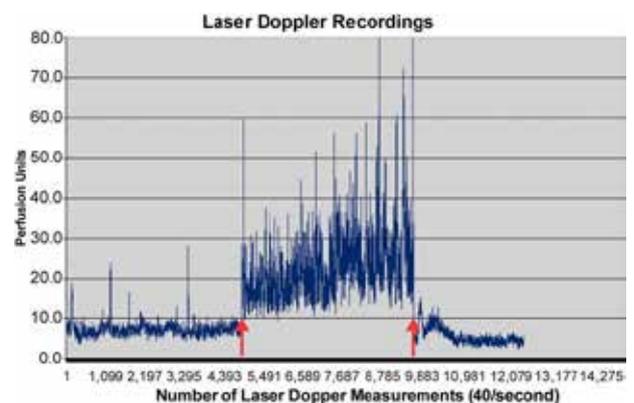
According to the laser Doppler readings, perfusion was found to be increased in some areas and reduced in others following application of NPWT, despite the use of the same suction pressure throughout. Yet the suction pressure used ( $-125$  mmHg) is traditionally known to increase perfusion fourfold.<sup>10</sup> Similar changes were seen when manual pressure was applied to the dressing without suction.

These findings are in keeping with the hypotheses proposed earlier. If tissue pressure were high enough (and this varies in different tissues for the same suction pressure), then the laser Doppler would record a reduced perfusion. This represents a true reduction in perfusion and most likely results from capillaries being totally occluded, with no increase in blood velocity or perfusion within them. If tissue pressure is minimally increased but not enough to totally occlude capillaries, then the laser Doppler records an increase in perfusion due to the increased velocity in the partially occluded capillaries. The latter is *not* a true increase in perfusion.

Perhaps the most important observation in this study was the fact that it was possible to elicit an apparent increase in perfusion by applying manual pressure to the dressing. This was possible in half of the cases tested. This shows the design flaw of this modality in NPWT research, as applying any amount of manual pressure to living tissue should never be expected to increase perfusion. This is a fundamental physiological principle and is the very reason pressure sores exist and why pressure bandages are applied to bleeding wounds. The fact that the laser Doppler could record increased perfusion in the face of tissue compression supports the hypothesis that the recordings of the laser Doppler may be misleading. In these circumstances, when vessels are compressed, the laser Doppler



**Figure 2:** Laser Doppler trace in the study of Morykwas et al. showing fourfold increase in perfusion during intermittent application of negative-pressure wound therapy (NPWT) at  $-125$  mmHg. (Reprinted with permission from Morykwas et al.<sup>10</sup>)



**Figure 3:** Increase in perfusion recorded by a laser Doppler during application of manual pressure to the sensor probe (2 minutes, baseline; 2 minutes, pressure; 85 seconds, no pressure). Two arrows indicate the points where manual pressure was applied and released, respectively.

reading is a reflection of blood velocity rather than perfusion, and an increase in velocity *can* be associated with a reduction in perfusion.

A potential argument against this hypothesis, however, is that the manual pressure was likely to have produced a reduction in perfusion beneath the dressing, resulting in the tissue, which is a centimeter or so away, experiencing an increase in perfusion due to a reactive hyperemia. If this argument were true, it would be in favor of the laser Doppler being a true measure of perfusion. However, this theory is invalidated by an informal study that was previously conducted. In the latter, it was hypothesized that one could emulate the perfusion increase seen in the laser Doppler traces of Morykwas et al.<sup>10</sup> (Figure 2) by merely applying pressure to the sensor.

A simple test was conducted where perfusion was recorded for 2 minutes, followed by manual pressure *directly on the sensor* for 2 minutes, and then no pressure again thereafter. It was shown that it was possible to obtain an apparent mean increase in perfusion from 7.3 (+1.8) perfusion units to 22.3 (+8.4) perfusion units, which then reduced to 5.6 (+2.2) perfusion units again when the pressure was released (Figure 3).

In the latter experiment, the increase in perfusion cannot be explained by the aforementioned argument relating to hyperemia. Tissues being compressed directly beneath the sensor cannot be hyperemic during the time of compression, as this would imply that they have a higher perfusion than prior to compression. The fact that it was possible to elicit an apparent increase in perfusion (according to the laser Doppler) when manual pressure was applied directly to the sensor supports the theory that the increased perfusion observed is due to vessel compression with increased velocity, rather than hyperemia.

Another potential argument against the central hypothesis is that when manual pressure was applied to the dressings, the capillary blood may have been forced away from the skin beneath the dressing (like water being forced out of a wet sponge if pressure were applied to it). This increase in flow away from the dressing would be recorded as an increase in perfusion by a laser Doppler probe next to the dressing, as the laser Doppler does not distinguish direction of flow. This would nevertheless be a true increase in flow (albeit in the opposite direction) and could explain the findings of the manual pressure studies (where an increase in perfusion was recorded).

However, if this was the reason for the recorded increase in perfusion following manual pressure to the dressing, rather than the hypothesis of vessel compression (with increased velocity), this would not explain why perfusion could be shown to increase when manual pressure was applied directly to the probe in the informal study. Only the hypothesis presented in this study relating to increased velocity can adequately explain that finding, and all of the other observations.

The findings of this study therefore appear to confirm the hypotheses that the laser Doppler is a flawed modality for measuring perfusion in the setting of NPWT. The implications of this are far-reaching as it implies that all prior studies on NPWT and perfusion that utilized laser Doppler are invalid. This does not, however, imply that these studies were poorly designed or of a low standard but merely that these researchers were misled by this device. It was not merely giving inaccurate values—it was demonstrating changes in perfusion that were the opposite of what actually occurred.

The evidence presented in this study explains many of the findings of studies that used laser Doppler previously. For example, the Lund group found that perfusion is reduced in close proximity to the NPWT dressing yet increased a couple of centimeters away.<sup>14–18</sup> The capillaries in close proximity to the NPWT were likely to be totally occluded, resulting in the laser Doppler correctly interpreting this as a reduction in perfusion, while those a couple of centimeters away are partially occluded, resulting in the laser Doppler incorrectly recording an increased perfusion in this area.

Furthermore, the fact that the zone of hypoperfusion that was seen in the studies of the Lund researchers<sup>17,18</sup> increased in size when suction pressure was increased is also in keeping with the findings of this study. As suction increased, so too did tissue pressure, resulting in a larger zone of totally occluded capillaries surrounding the dressing. In these totally occluded capillaries, there was no blood

velocity as perfusion had ceased and, as such, the laser Doppler correctly recorded a reduced perfusion.

The outcomes of this study also explain the conflicting findings that have been published regarding what the optimal suction pressures are. Morykwas et al. demonstrated that perfusion is maximally increased at  $-125$  mmHg and reduced at  $-400$  mmHg.<sup>10</sup> Timmers et al., on the other hand, demonstrated that perfusion is increased with suction pressures as high as  $-500$  mmHg.<sup>11</sup> Morykwas et al. were experimenting on pig wounds,<sup>10</sup> while Timmers et al. conducted their study on intact skin of the forearms of healthy volunteers.<sup>11</sup> The different consistency of porcine and human tissue is likely to result in different degrees of capillary compression for the same suction pressure, resulting in the very different perfusion readings of the laser Doppler in these studies.

It could be argued that the findings of an experiment conducted on intact skin are irrelevant to what occurs in an actual wound, which is where wound healing takes place. However, the focus of this study is not on what the specific perfusion of skin is and its effects on wound healing. Instead, a hypothesis is being tested to evaluate whether laser Doppler is an inappropriate tool to measure perfusion. Intact skin was considered an acceptable medium to test the physics discussed in this paper. All the factors being tested, namely increased tissue pressure and capillary flow, are present in intact skin. This study merely assesses how the laser Doppler recording is affected by changes in these variables, the findings of which deepen our understanding of its workings. Should the central hypothesis be accepted, then we know that one cannot use this device to measure perfusion during NPWT, whether on open wounds or intact skin. This fact is critical to our evaluation and further understanding of perfusion at the wound bed as many studies use this device to measure perfusion at the wound bed.

How can reduced perfusion possibly be good for a wound? This perplexing question needs to be discussed, as the answer is not immediately obvious. In most wounds, the application of NPWT at a reasonable suction pressure will result in a relative ischemia rather than total anoxia. It is well known that ischemia is one of the most powerful stimuli for angiogenesis, resulting in an increase in the transcription factor, hypoxia-inducible factor-1 $\alpha$ .<sup>27</sup> This transcription factor induces increased levels of, among others, vascular endothelial growth factor and angiopoietin. It up-regulates glycolysis, metabolic activity, antioxidant production, and erythropoiesis while enhancing cellular proliferation.<sup>27</sup> This serves to enhance the development of granulation tissue and the proliferation of cells involved in the wound healing process.

In addition, the increased tissue pressure, which was the cause of the reduced perfusion, is also beneficial, as it reduces edema and prevents further formation thereof.<sup>9</sup> Other factors such as macroand microdeformation with their effects on cellular mechanotransduction are likely to be significant role players too. “Mechanotransduction” refers to the many mechanisms by which cells convert mechanical stimuli into chemical activity, including, among others, synthetic activity.<sup>28–30</sup>

It can therefore be seen that the increased tissue pressure and the associated reduction in perfusion are likely to be beneficial to wound healing, after time has been allowed for the tissues to respond to this and providing they are not already severely hypoxic. Despite the fact that NPWT may increase vascularity at a later stage, its initial reduction in perfusion implies that it may be detrimental to severely hypoxic tissues. The proposal that NPWT may be beneficial to ischemic myocardium,<sup>5,12,13,20,22</sup> for example, needs to be cautiously reviewed. The potential for disastrous consequences in this scenario is a reality if borderline-perfused myocardium were rendered anoxic by the compressive effects of NPWT.

It must be mentioned, however, that there are studies where modalities other than laser Doppler were used to measure perfusion changes due to NPWT and where an increase in perfusion was shown.<sup>2,5,19</sup> However, in light of our better understanding of the biomechanics of NPWT, there may be potential flaws in the methodology of these studies and these need to be highlighted.

In the study of Lindstedt et al., it was shown that coronary artery blood flow is increased by NPWT using electromagnetic flow meter probes.<sup>5</sup> However, the probes were placed around the roots of three coronary arteries, which were not directly beneath the NPWT dressing. These areas of measurement were therefore not exposed to the compressive forces of the foam; on the contrary, they were exposed to the hypobaric pressures that were created within the chest cavity.<sup>4</sup> This may be an explanation for the observed increased perfusion in these vessels.

In the study of Ichioka et al.,<sup>19</sup> a very superficial wound was created on the gluteal region of a mouse, allowing the intact subdermal vascular plexus to be directly visualized with an "intravital microscope." The foam of the NPWT was significantly smaller than the wound and was applied to the intact tissue adjacent to the wound, while the wound itself was left covered only with the transparent adhesive drape.

The small size of the foam and its relative distance from the vasculature being observed reduces the likelihood that any compressive forces will be transmitted to them or reduce their flow. The contraction of the foam, however, may have been enough to stretch the surrounding tissues, including those within the wound, thereby enlarging the vessels' caliber and increasing the flow.<sup>19</sup> This dressing configuration is not representative of a typical NPWT dressing on a wound, nor does it possess the biomechanical properties of a dressing comprising of a relatively larger portion of foam; this may account for the findings of increased perfusion.

Lastly, Chen et al. used a microcirculation microscope to demonstrate increased perfusion adjacent to wounds created on rabbit ears.<sup>2</sup> Unlike the previous two studies, this was a true representation of a typical wound within soft tissues undergoing NPWT. An increase in perfusion was shown 0.5 cm from the wound edge at pressures as high as 150 mmHg. In the context of the current study, the findings of Chen et al. are difficult to explain. The following explanation may be considered however. In a typical wound undergoing NPWT, the depth is sufficient to allow the foam to apply force to the outer walls of the wound, resulting in increased tissue pressure adjacent to this.<sup>6-9</sup> Creating a wound by removing the thin skin of a rabbit

ear results in a wound so shallow that there is very little if any outer wound "wall" for the foam to apply force to. Therefore, little pressure will be transmitted to the tissues 0.5 cm away from this contact area. As in the study of Ichioka et al., the contracting foam (and its resultant stretching of surrounding vasculature) may result in increased perfusion, with the minuscule forces applied to the wound walls doing little to counteract this.

In conclusion, sufficient evidence has been shown in this study to suggest that laser Doppler velocimetry is an inappropriate modality to measure perfusion in NPWT. Its flawed measuring technique when used in the setting of NPWT has resulted in the misconception that NPWT immediately increases perfusion. Not only has this led to potentially inappropriate indications but also confounded our understanding of its mechanism of action. The findings and subsequent discussion of this study explain many of the inconsistencies of prior perfusion studies.

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## Conflict of Interest

The authors have no financial conflict of interest to declare.

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